(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 July 2001 (26.07.2001)

PCT

(10) International Publication Number
WO 01/53457 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US01/01665

(22) International Filing Date: 18 January 2001 (18.01.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/489,219 21 January 2000 (21.01.2000) US

(71) Applicant: UNIVERSITY OF CONNECTICUT HEALTH CENTER [US/US]; 263 Farmington Avenue, Farmington, CT 06030 (US).

(72) Inventor: SRIVASTAVA, Pramod, K.; 70 Pheasant Run, Avon, CT 06001 (US).

(74) Agents: ANTLER, Adriane, M. et al.; Pennie & Edmonds LLP, 1155 Avenue of the Americas, New York, NY 10036 (US).

(81) Designated States *(national)*: AU, CA, JP.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/53457 A2

(54) Title: VACCINES AGAINST NEURODEGENERATIVE DISORDERS

(57) Abstract: The present invention relates to pharmaceutical compositions comprising antigenic molecules for use as vaccines for the treatment and prevention of neurodegenerative disorders and diseases, such as Alzheimer's Disease. The invention further relates to methods for the use of such pharmaceutical compositions as immunotherapeutic agents for treating and protecting against such neurodegenerative disorders and disease.

VACCINES AGAINST NEURODEGENERATIVE DISORDERS

This invention was made with government support under grant numbers CA44786 and CA64394 awarded by the National Institutes of Health. The government has certain rights in the invention.

1. INTRODUCTION

The present invention relates to compositions and methods for the use of antigenic peptides associated with neurodegenerative disorders as vaccines against neuropsychiatric disorders, such as Alzheimer's Disease.

2. BACKGROUND OF THE INVENTION

Neurodegenerative disorders, such as Alzheimer's Disease (AD), are currently the fourth leading cause of death in developed countries. At present, there is no known cure or method for their prevention. However, there is a great effort underway to find new ways for treating patients with neurodegenerative disorders, and for preventing neurodegenerative disorders, such as AD, in those at high risk for these diseases.

Neuropsychiatric and neurodegenerative disorders are beginning to be understood at the molecular level. For example, mutations or polymorphisms in four genes are known that give rise to an increased risk of, or early onset of, AD: amyloid precursor protein (APP), the precurser to Aβ (Goate *et al.*, 1991, Nature 349: 704-706); an allele of apolipoprotein, ApoE4; presenilin-1 (PS1; Sherrington *et al.*, 1995, Nature 375: 754-760) ; and presenilin-2 (PS2; Levy-Lahad *et al.*,1995, Science 269: 973-977). Mutations in each of these four genes can have the same result -- an increased production of the 42-43 amino acid form of Aβ. APP, a single transmembrane domain integral membrane glycoprotein, is processed via alternative proteolytic pathways. In one pathway, APP molecules are cleaved within the Aβ sequence by a plasma membrane protease, resulting in secretion of the ectodomain of APP. In an alternative pathway, Aβ peptides are generated by endoproteolytic cleavage of APP. Mutations in APP can result in the shift of the processing of the APP precurser so that more of the 42 or 43 amino acid form of Aβ is produced. PS1 and PS2 are highly homologous 43 to 50 kD proteins with eight transmembrane domains. Presenilin polypeptides acculmulate as 27/28 kDa N-terminal and 16/17 kDa C-terminal kDa derivatives that become stably associated with each other *in vivo*. Most genetic abnormalities in the presenilin are missense mutations that result in single amino acid substitutions. Unlike the case of early onset of AD, no specific gene mutations are

associated with the inheritance in the case of late onset AD. However, specific alleles of apolipoprotein E and α-2 macroglobulin are associated with increased risk for AD (Blacker *et al.*, 1998, Nature Genet. 19: 357-360).

Likewise, the molecular basis of neurodegenerative diseases other than AD are also beginning to be understood. Strikingly similar pathologies commonly associated with the neurodegenerative disorders, can be arrived at by a large number of different genetic mechanisms. For example, a pathogenic mutation in the prion gene results in both tangle and Lewy body pathologies of prion disease (Feany and Kickson, 1995, Am. J. Pathol. 146: 1388). Mutations in tau protein lead to NFTs and dementia in frontotemporal dementia (Hutton et al., 1998, Nature 393: 702), mutations in synuclein lead to the presence of Lewy bodies and Parkinson's disease (Polymeropoulos *et al.*, 1997, Science 276: 2045).

Neurodegenerative disorders are typically characterized by a number of neuropathological abnormalities, such as neuritic plaques, neurofibrillary tangles (NFTs), Lewy bodies, and intranuclear inclusions. For example, Alzheimer's Disease, the most common cause of dementia, is characterized pathologically by neurodegeneration, the presence of large numbers of amyloid plaques, and neurons that accumulate tau and ubiquitin reactivities within NFTs. The result is damage to regions of the brain and neural circuits responsible for memory and cognition, including neurons in the neocortex, hippocampus, amygdala, basal forebrain cholinergic system, and brainstem monoaminergic nuclei.

Neuritic plaques are extracellular lesions, consisting largely of a deposit of a peptide called β-amyloid (Aβ). The predominant forms of β-amyloid are the 40 amino-acid form, Aβ40, and the 42 amino acid form, Aβ42. Neurofibrillary tangles are intracellular lesions consisting of poorly soluble filaments of the protein tau. In particular, hyperphosphorylated forms and highly ubiquitinated forms of the tau protein, a microtubule-binding protein, are predominant in such NFTs. Tangles are found in frontotemporal dementia associated with Parkinson's disease, Alzheimer's Disease, progressive supranuclear palsy, Guam disease, and some forms of prion disease. Lewy bodies, consisting largely of the protein α-synuclein, are found in Parkinson's disease, some forms of Alzheimer's disease, and Lewy body dementia (for review, see Hardy and Hardy, 1998, Science 282: 1075-1079 and Greefnfield's Neuropatholgy, Graham and Lantos (Eds.), Arnold, London, 1997).

Intranuclear inclusions are often associated with an unstable triplet repeat mutation (polyCAG) which cause polyglutamine diseases. The presence of the repeat, which encodes polyglutamine, in the gene encoding huntingtin, has been shown to cause

intranuclear polyglutamine inclusions which typify Huntington's Disease (Davies *et al.*, 1997, Cell 90: 537; Davies *et al.*, 1998, Lancet 351: 131)

The increase in understanding about the underlying basis for neuropsychiatric and neurodegenerative disorders, such as AD, have led to the search for cures and treatments for these diseases. Immunization of mice with Aβ antigens was shown to prevent the formation of amyloid plaques in the mice (Schenk *et al.*, 1999, Nature 400:173-177; see also St. George-Hyslop and Westaway, 1999, Nature 400:116-117). In humans, it has been noted that hormone replacement therapy that provides estrogen to post-menopausal women provides a beneficial effect to the development of AD, although the benefits are determined by the genetic background of the individual (van Duijn, 1999, Maturitas 31:201-205). Additionally, since the cognitive impairment associated with AD is at least in part attributed to cholinergic deficits, several acetylcholinesterase inhibitors (AchEIs), for example tacrine hydrochloride (sold under the trademark COGNEX), have been used in an attempt to prevent the degradation of acetylcholine and retard AD-associated neurodegeneration, the use having met with only moderate success (Krall et al., 1999, Ann. Pharmacother. 33:441-450). Other treatments for AD are currently being tested in clinical trials, for example anti-inflammatory drugs and α-tocopherol (Shadlen and Larson, 1999, Postgrad. Med. 105:109-118).

These references, however, have not described the use of defined antigenic peptides, other than β amyloid, as vaccines for treatment or prevention of neurodegenerative disorders. Vaccination has been used against infectious diseases such as polio, tetanus, chicken pox, measles, *etc.*, and has eradicated these diseases in many countries of the world. Such vaccination with non-live materials such as proteins generally leads to an antibody response or CD4+ helper T cell response (Raychaudhuri and Morrow, 1993, Immunology Today 14: 344-348). On the other hand, vaccination or infection with live materials such as live cells or infectious viruses generally leads to a CD8+ cytotoxic T-lymphocyte (CTL) response. Methods for the use of antigenic peptides associated with nuerodegenerative disorders as vaccines to generate an immune response against such neurodegenerative disorders would greatly increase the prospects of treating and preventing such disorders.

Citation or discussion of a reference herein shall not be construed as an admission that such is prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention provides compositions and methods for the use of antigenic peptides associated with neurodegenerative disorders, such as Alzheimer's Disease, as vaccines for the treatment and prevention of such disorders.

The invention provides a pharmaceutical composition comprising comprising a pharmaceutically acceptable carrier and an immunogenic amount of an antigenic molecule effective for treatment of or prevention of a neurodegenerative disorder, which antigenic molecule displays the antigenicity of an antigen associated with a neurodegenerative disorder, with the proviso that the antigenic molecule is not β amyloid. In one embodiment, the antigenic molecule is purified. In another embodiment, the pharmaceutical carrier is an adjuvant. In another embodiment, the antigenic molecule is purified. In another embodiment, the antigenic molecule is purified to apparent homogeneity, as viewed on an SDS-PAGE gel. Preferably, the ND-associated hsp-peptide complexes of the invention are used in purified form, preferably to apparent homogeneity as viewed on an SDS-PAGE gel, or to at least 60%, 70%, 80%, or 90% of total protein.

The invention further provides a pharmaceutical composition comprising an amount of an antigenic molecule and one or more pharmaceutically acceptable carriers effective for treatment of or prevention of a neurodegenerative disorder, which antigenic molecule displays the antigenicity of an antigen associated with a neurodegenerative disorder, with the proviso that the antigenic molecule is not β amyloid. In one embodiment, the one or more carriers is an adjuvant. In a specific embodiment, the adjuvant is a mineral salt, a mineral salt gel, an immunostimulatory molecule, a particulate or microparticulate adjuvant, or a mucosal adjuvant. The mineral salt may be an aluminum hydroxide, aluminum phosphate, or calcium phosphate. Immunostimulatory molecules may be a cytokine, saponin, muramyl dipeptide or tripeptide derivative, CpG dinucleotide, CpG oligonucleotide, monophosphoryl Lipid A , or a polyphosphazene. Particulate adjuvants may be emulsions, liposomes, virosomes, cochleates, or an immune stimulating complex.

In one embodiment, the pharmaceutical composition comprises at least two carriers selected from the group consisting of a mineral salt, a mineral salt gel, an immunostimulatory molecule, a particulate adjuvant, a microparticulate adjuvant, and a mucosal adjuvant. In another embodiment, the carrier is covalently associated with the antigenic molecule. In another embodiment, the antigenic molecule is coupled to a universal helper. In another embodiment, the antigenic molecule is selected from the group consisting of, an ApoE4-Aβ complex, tau protein, a mutant amyloid precurser protein, a mutant of presenillin, α-synuclein, an oligomeric Aβ complex, and a prion protein, or a fragment thereof.

The invention further provides recombinant cells useful for the prevention and treatment of neurodegenerative disorders. In one embodiment, the invention provides a recombinant cell transformed with a nucleic acid comprising a nucleic acid sequence that is operably linked to a promoter, said nucleic acid sequence encoding a fusion protein that comprises an antigenic molecule operatively linked to a carrier protein, which antigenic molecule displaying the antigenicity of an antigen associated with a neurodegenerative disorder. The invention further provides a pharmaceutical composition comprising such a recombinant cell.

In another embodiment, a recombinant cell is provided, which recombinant cell is transformed with (i) a first nucleic acid comprising a first nucleotide sequence that is operably linked to a first promoter and encodes an antigenic molecule that displays the antigenicity of an antigen associated with a neurodegenerative disorder, and (ii) a second nucleic acid comprising a second nucleic acid sequence that is operably linked to a second promoter and encodes a carrier protein, such that the antigenic molecule and the carrier protein are expressed within the cell and non-covalently associate with each other to form a complex that in sufficient amount is capable of eliciting an immune response to the antigenic molecule. The invention further provides a pharmaceutical composition comprising such a recombinant cell.

In a specific embodiment, the recombinant cell is a human cell. In another embodiment, the antigenic molecule of the recombinant cell is an ApoE4-Aβ complex or a fragment thereof, tau protein or a fragment thereof, a mutant amyloid precurser protein or a fragment thereof, a mutant of presenillin or a fragment thereof, α-synuclein or a fragment thereof, a prion protein or a fragment thereof, or an oligomeric Aβ complex.

The invention further provides a method for preparing a fusion protein capable of eliciting an immune response against a neurodegenerative disorder, said method comprising: a) culturing a recombinant cell transformed with a nucleic acid comprising a nucleotide sequence that is operably linked to a promoter and that encodes a fusion protein comprising an antigenic molecule operatively linked to a carrier, which antigenic molecule displays the antigenicity of an antigen associated with a neurodegenerative disorder with the proviso that the antigenic molecule is not β amyloid, under conditions such that the fusion protein is expressed by the cell; and b) recovering the fusion protein from the cells. In one embodiment, the one or more antigenic molecules is an ApoE4-Aβ complex or a fragment thereof, tau protein or a fragment thereof, a mutant amyloid precurser protein or a fragment thereof, a mutant of presenillin or a fragment thereof, α-synuclein or a fragment thereof, an oligomeric Aβ complex, or a prion protein or a fragment thereof.

The invention further provides a method for mixing the carrier with one or more antigenic molecules *in vitro*, which one or more antigenic molecules display the antigenicities of antigens associated with a neurodegenerative disorder, said method comprising: a) incubating the antigenic molecule or molecules with a carrier protein under conditions and for a length of time sufficient for formation of the complex, and b) isolating said complexes. In one embodiment, the one or more antigenic molecules is an ApoE4-Aβ complex or a fragment thereof, tau protein or a fragment thereof, a mutant amyloid precurser protein or a fragment thereof, a mutant of presenillin or a fragment thereof, α-synuclein or a fragment thereof, an oligomeric Aβ complex, or a prion protein or a fragment thereof.

The invention further provides a method for eliciting an immune response against neurodegenerative disorder–associated antigenic peptides. In one embodiment, a method is provided for eliciting an immune response against an antigen associated with a neurodegenerative disorder in an individual comprising administering to the individual an antigenic molecule that displays the antigenicity of an antigen associated with a neurodegenerative disorder, with the proviso that the antigenic molecule is not β amyloid, and one or more pharmaceutically acceptable carriers in an amount effective to elicit an immune response. In another embodiment, the method further comprises, before, concurrently, or after administration of the immunogenic complex, administering to the individual a composition comprising antigen presenting cells sensitized *in vitro* with a sensitizing amount of a second antigenic molecule, in which said second antigenic molecule shares at least one antigenic determinant with the first antigenic molecule.

In one embodiment, a method is provided for treating or protecting against a neurodegenerative disorder in an individual having a neurodegenerative disorder, or in whom prevention of a neurodegenerative disorder is desired, comprising administering to the individual an antigenic molecule and a pharmaceutically acceptable carrier in an amount effective to treat or protect against said neurodegenerative disorder, wherein said antigenic molecule displays the antigenicity of an antigen associated with said neurodegenerative disorder, with the proviso that the antigenic molecule is not β amyloid. In one embodiment, the method further comprises, before, concurrently or after administration of the immunogenic complex, administering to the individual a composition comprising antigen presenting cells sensitized *in vitro* with a sensitizing amount of a second antigenic molecule, said second antigenic molecule sharing at least one antigenic determinant with the first antigenic molecule.

In another embodiment, a method is provided for treating or protecting against a neurodegenerative disorder in a subject having a neurodegenerative disorder or in

whom prevention of a neurodegenerative disorder is desired comprising: a) culturing a recombinant cell transformed with a nucleic acid comprising a nucleotide sequence that is operably linked to a promoter and that encodes a fusion protein comprising an antigenic molecule and a carrier, which antigenic molecule displays the antigenicity of an antigen associated with a neurodegenerative disorder, under conditions such that the fusion protein is expressed by the cell; b) recovering the fusion protein from the cells; and c) administering to the subject an amount of the fusion protein effective to treat or protect against the neurodegenerative disorder. In one embodiment, the antigenic molecule is an ApoE4-Aβ complex or a fragment thereof, tau protein or a fragment thereof, a mutant amyloid precurser protein or a fragment thereof, a mutant of presenillin or a fragment thereof, α-synuclein or a fragment thereof, an oligomeric Aβ complex, or a prion protein or a fragment thereof.

Neurodegenerative disorders that can be prevented or treated using the compositions and methods of the invention include disorders relating to the central nervous system and/or peripheral nervous system including, but not limited to, cognitive and neurodegenerative disorders such as Alzheimer's Disease, age-related loss of cognitive function and senile dementia, Parkinson's disease, amyotrophic lateral sclerosis, Wilson's Disease, cerebral and progressive supranuclear palsy, Guam disease, Lewy body dementia, prion diseases, such as spongiform encephalopathies, *e.g.*, Creutzfeldt-Jakob disease, polyglutamine diseases, such as Huntington's disease, myotonic dystrophy, Freidrich's ataxia and other ataxias, well as Gilles de la Tourette's syndrome, seizure disorders such as epilepsy and chronic seizure disorder, stroke, brain or spinal cord trauma, AIDS dementia, alcoholism, autism, retinal ischemia, glaucoma, autonomic function disorders such as hypertension and sleep disorders, and neuropsychiatric disorders that include, but are not limited to schizophrenia, schizoaffective disorder, attention deficit disorder, dysthymic disorder, major depressive disorder, mania, obsessive-compulsive disorder, psychoactive substance use disorders, anxiety, panic disorder, as well as unipolar and bipolar affective disorders. Additional neuropsychiatric and neurodegenerative disorders include, for example, those listed in the American Psychiatric Association's Diagnostic and Statistical manual of Mental Disorders (DSM), the most current version of which is incorporated herein by reference in its entirety.

4. DETAILED DESCRIPTION OF THE INVENTION

The invention provides compositions comprising antigenic peptides associated with neurodegenerative diseases and disorders, such as Alzheimer's Disease

(AD), which can be used as vaccines to protect against and/or treat such diseases and disorders.

In certain embodiments, the compositions and formulations of the present invention are administered to a human subject to prevent a neurodegenerative disorder (ND), including inhibiting the progression of a disease in an asymptomatic patient, for example a patient having the molecular landmarks of AD (*e.g.*, above normal levels of phosphorylated Tau). In a preferred embodiment, the human subject to which the vaccines of the invention are administered is one having a genetic background that increases the likelihood of a given ND (*e.g.* for Alzheimer's Disease, having the E4 allele of Apolipoprotein E or having a mutation in APP, PS1 or PS2 which gives rises to FAD). In another embodiment, the human subject to which the preventative vaccines of the invention are administered is a non-senile adult above the age of 60.

In other embodiments, the compositions and formulations of the present invention are administered to a human subject that has been diagnosed with a ND or suspected of having a ND. According to the present invention, treatment of a ND encompasses the treatment of patients already diagnosed as having ND at any clinical stage; the prevention of the disease in the patients with early symptoms and signs; the delay of the onset or evolution or aggravation or deterioration of the symptoms or signs of a ND; and/or promoting regression of a ND in symptomatic patients.

Antigenic peptides that can be used to form such antigenic peptide complexes are peptides found in the brains of patients with neurodegenerative disorders, or synthesized based on the known sequences of peptides associated with the pathology of such disorders. Such peptides include, but are not limited to, peptides of, or derived from, the following: tau protein, which is found in neurofibrillary tangles (NFTs); α-synuclein, which is found in Lewy bodies; prion protein (PrP), which is associated with spongiform encephalopathies, *e.g.*, Creutzfeldt-Jakob disease, and other peptides associated with such neuropsychiatric disorders. Such antigenic peptides are described in detail in Section 4.1, below.

ND-associated peptide antigens (and ND-associated peptide antigen – carrier protein complexes) can be produced by any of a number of methods. For example, antigenic peptides associated with neurodegenerative disorders can be obtained by recombinant or synthetic methods, or can be isolated and purified from recombinant cells.

4.1 Neurodegenerative Disease Antigens

Peptide antigens associated with neurodegenerative diseases and disorders (ND), herein termed "ND-associated antigens", comprise peptides and polypeptides, and fragments thereof, that are found associated with plaques, tissues or cells of subjects with neurodegenerative diseases and disorders, such as Alzheimer's Disease, and that are specific to subjects with such diseases and disorders. ND-associated antigens include, but are not limited to, fragments of ApoE4-Aβ complexes, hyperphosphorylated tau and fragments thereof, APP mutant proteins and fragments thereof, presenillin mutant proteins and fragments thereof, α-synuclein of Lewy bodies and fragments thereof, prion protein (PrP) and fragments thereof, oligomeric Aβ complexes, as well as other antigenic peptides present in ND plaques, neurofibrillary tangles and lesions, cells, and tissues. Such antigenic ND-associated peptides may be produced by any synthetic or recombinant means known in the art. The compositions of such peptides and the methods for their production, isolation, or synthesis are described in detail hereinbelow.

4.1.1 ND-Associated Antigenic Molecules

Antigenic peptides associated with nueropsychiatric disease or neurodegenerative disorders, or antigenic portions thereof, can be chosen from among those known in the art to be associated with such diseases and disorders. Alternatively, such antigens can be selected for their antigenicity or their immunogenicity, as determined by immunoassays or by their ability to generate an immune response.

In one embodiment, apolipoproteins, or fragments thereof, can be used as antigens. Apolipoprotein E (ApoE) is present in amyloid plaques in AD (Namba *et al.*, 1991, Brain Res. 541: 163-166). One isoform of ApoE, ApoE4, is particularly associated with a high risk for AD. Thus, complexes of ApoE4, or derivatives, fragments, or analogs thereof, can be sources for antigenic peptides. In addition, complexes of apolipoproteins and Aβ, or fragments such as proteolytic products thereof, can also be used as antigens.

In various embodiments, antigenic peptides may comprise amino acid sequences derived from APP proteins known to be associated with AD, or fragments thereof. In a specific embodiment, peptide fragments of a mutant APP comprising a mutation at codon 717 may be used (Chartier-Harlin *et al.*, 1991, Nature 353: 844-6). In another specific embodiment, antigenic molecules may be peptide fragments of a mutant APP comprising a mutation at codon 670 or 671.

In another embodiment, antigenic peptides derived from tau protein sequences are used. Hyperphosphorylated and ubiquitinated forms of the microtubule-associated protein tau, as well as tau mutations, are found associated with the pathologies of

many neurodegenerative disorders, including AD (Lynch *et al.*, Neurology 44: 1878-1884; Spillantini *et al.*, 1998, Proc. Natl. Acad. Sci. U.S.A. 95: 7737-41). Thus, tau protein-derived antigenic peptides may be used to elicit an immune response against such neurodegenerative disorders. The gene sequence encoding tau protein and the corresponding proteins sequences are available in public databases (GenBank accession no. NM_005910). In addition, modified amino acid sequences derived from hyperphosphorylated or ubiquitinated tau protein are also within the scope of the invention.

In another embodiment, antigenic peptides may comprise amino acid sequences derived from presenilin mutant proteins, PS1 (GenBank accession no. NM_000021; Sherrington *et al.*, 1995, Nature 375: 754-760) and PS2 (GenBank accession no. 000447 ; Levy-Lahad *et al.*, 1995, Science 269: 973-977).

In another embodiment, antigenic peptides may comprise amino acid sequences derived from α-synuclein (GenBank accession no. AF044672), which is found in Lewy bodies in various neurodegenerative disorders. Nucleotide sequences encoding α-synuclein amino acid sequences may be used to for recombinant expression of antigenic α-synuclein peptide fragments. Such α-synuclein antigenic molecules may be used to treat a subject with a neurodegenerative disorder having the pathology of the presence of Lewy bodies. Such disorders include, but are not limited to, Parkinson's disease, some forms of Alzheimer's disease, and Lewy body dementia.

In another embodiment, antigenic peptides may comprise amino acid sequences derived from the known amino acid sequence of prion protein (PrP; GenBank accession no. AF076976; GenBank accession no. NM_000311, Kretzschmar *et al.*, 1986, DNA 5: 315-324). Such prion antigenic molecules may be used to treat a subject with a neurodegenerative disorder having a prion pathology. Such disorders include, but are not limited to, spongiform encephalopathies, *e.g.*, Creutzfeldt-Jakob disease.

In another embodiment, antigenic peptides may comprise amino acid sequences derived from the huntingtin protein sequences (GenBank accession no. NM_002111), and mutants, variants or fragments thereof. Such antigenic peptides can be used in vaccine formulations to treat subjects with Huntington's Disease, or any other disease associated with altered activity or expression of huntingtin. In another embodiment, polyglutamine repeats, as well as proteins containing polyglutamine repeats are used as antigenic peptides.

In another embodiment, an antigen may comprise a dimeric, oligomeric or multimeric form of a β-amyloid. In particular, fibrils can be formed by *in vitro* polymerization of Aβ40 and Aβ42 (Harper and Lansbury, 1997, Annu. Rev. Biochem. 66: 385-407). The Aβ amyloid protofibril is a discrete intermediate in the *in vitro* process of

Aβ fibril formation (Harper *et al.*, 1997, Chem. Biol. 4: 119-125). This stable intermediate, and proteolytic fragments thereof, can also be used as antigenic peptides for use in therapeutic vaccines.

If the amino acid sequence of an ND-associated antigenic peptide is not yet known, it may be determined either by manual or automated amino acid sequencing techniques well known in the art. However, amino acid sequences and nucleotide sequences of antigenic molecules described above are generally available in sequence databases, such as GenBank. Computer programs, such as Entrez, can be used to browse the database, and retrieve any amino acid sequence and genetic sequence data of interest by accession number. These databases can also be searched to identify sequences with various degrees of similarities to a query sequence using programs, such as FASTA and BLAST, which rank the similar sequences by alignment scores and statistics.

4.1.2. ND-Associated Antigen–T Cell Epitope Fusions

To increase the immunogenicity of an ND-associated antigenic peptide, the ND-associated antigenic peptide may be coupled to a carrier protein comprising one or more T cell epitopes. Such "universal" T helper cell determinants can enhance immunogenicity of peptide constructs containing a limited number of epitopes. For example, because mycobacteria can induce a strong T-cell response, mycobacteria derivatives have been used to potentiate immune responses (Lachmann *et al.*, eds., 1986, in *Ciba Foundation Symposium on Synthetic Peptides as Antigens*, Wiley, Chichester, Vol. 119, pp. 25-57). In one embodiment, antigens can be conjugated to a tuberculin purified protein derivative (PPD), and used to immunize subjects, optionally primed with BCG (bacillus Calmette-Guerin *Mycobacterium tuberculosis* var. *bovis*). In another embodiment, tetanus toxin sequences can be used as carrier proteins, for example, tt830-844 from tetanus toxin (Kumar *et al.*, 1992, J. Immunol. 148: 1499-1505). In yet another embodiment, the circumsporozoite protein CST3 of a human malarial parasite, *Plasmodium falciparum* (Kumar *et al.*, supra), may be coupled to an ND-associated antigen to enhance immunogenicity.

4.1.3 Synthetic Production of ND-Associated Antigens

Once the sequence of an ND-associated peptide antigen has been determined or obtained, the peptide can be produced, either by recombinant techniques or by synthetic methods. The antigenic peptide may be synthesized using conventional peptide synthesis or any of a number of other protocols well known in the art. For example, a peptide corresponding to a mutant protein associated with AD, such as an APP or presenilin mutant,

can be synthesized by use of a peptide synthesizer. Either the entire protein can be synthesized, or an antigenic determinant thereof, preferably the portion of the protein that contains the mutant or variant amino acid(s).

An ND-associated peptide potentially useful for a vaccine protective against neurodegenerative diseases and disorders may be synthesized by using conventional peptide synthesis or other protocols well known in the art. Peptides having the same amino acid sequence as peptides associated with ND-associated diseases and disorders may be synthesized by solid-phase peptide synthesis using procedures similar to those described by Merrifield, 1963, J. Am. Chem. Soc. 85: 2149. During synthesis, N-α-protected amino acids having protected side chains are added stepwise to a growing polypeptide chain linked by its C-terminal and to an insoluble polymeric support *i.e.*, polystyrene beads. The peptides are synthesized by linking an amino group of an N-α-deprotected amino acid to an α-carboxyl group of an N-α-protected amino acid that has been activated by reacting it with a reagent such as dicyclohexylcarbodiimide. The attachment of a free amino group to the activated carboxyl leads to peptide bond formation. The most commonly used N-α-protecting groups include Boc which is acid labile and Fmoc which is base labile. Details of appropriate chemistries, resins, protecting groups, protected amino acids and reagents are well known in the art (see Atherton *et al.*, 1989, Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, and Bodanszky, 1993, Peptide Chemistry, A Practical Textbook, 2nd Ed., Springer-Verlag).

In addition, analogs and derivatives of ND-associated antigenic peptide proteins can be chemically synthesized. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the sequence of the ND-associated antigenic peptide. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ-Abu, ϵ-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general.

Purification of the resulting peptides is accomplished using conventional procedures, such as preparative HPLC using gel permeation, partition and/or ion exchange chromatography. The techniques, choice of appropriate matrices and buffers are well known in the art (Atherton *et al.*, 1989, Solid Phase Peptide Synthesis: A Practical Approach, IRL Press).

4.1.4 Recombinant Production of ND-Associated Antigens

As an alternative to synthetic production, ND-associated antigenic peptides and polypeptides, such as those mentioned in Section 4.1.1, above, can produced by recombinant means. Once the nucleotide sequence encoding an ND-associated antigen has been identified, the nucleotide sequence, or a fragment thereof, can be obtained and cloned into an expression vector for recombinant expression. The expression vector can then be introduced into a host cell for propogation of the antigen. Methods for recombinant production of ND-associated peptide antigens are described in detail herein.

The DNA may be obtained by DNA amplification or molecular cloning directly from a tissue, cell culture, or cloned DNA (*e.g.*, a DNA "library") using standard molecular biology techniques (see *e.g.*, Methods in Enzymology, 1987, volume 154, Academic Press; Sambrook *et al.* 1989, Molecular Cloning - A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, New York; and *Current Protocols in Molecular Biology*, Ausubel et al. (eds.), Greene Publishing Associates and Wiley Interscience, New York, each of which is incorporated herein by reference in its entirety). Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. Whatever the source, the ND-associated antigen gene should be cloned into a suitable vector for propagation of the gene.

In a preferred embodiment, DNA can be amplified from genomic or cDNA by polymerase chain reaction (PCR) amplification using primers designed from the known sequence of a related or homologous ND-associated antigen. PCR is used to amplify the desired sequence in DNA clone or a genomic or cDNA library, prior to selection. PCR can be carried out, *e.g.*, by use of a thermal cycler and Taq polymerase (sold under the trademark of GENE AMP). The polymerase chain reaction (PCR) is commonly used for obtaining genes or gene fragments of interest. For example, a nucleotide sequence encoding an ND-associated antigen of any desired length can be generated using PCR primers that flank the nucleotide sequence encoding the peptide-binding domain. Alternatively, an ND-associated antigen gene sequence can be cleaved at appropriate sites with restriction endonuclease(s) if such sites are available, releasing a fragment of DNA encoding the ND-associated antigen gene, or an antigenic derivative thereof. If convenient restriction sites are not available, they may be created in the appropriate positions by site-directed mutagenesis and/or DNA amplification methods known in the art (see, for example, Shankarappa *et al.*, 1992, PCR Method Appl. 1: 277-278). The DNA fragment that encodes the ND-associated antigen is then isolated, and ligated into an appropriate

expression vector, care being taken to ensure that the proper translation reading frame is maintained.

In an alternative embodiment, for the molecular cloning of an ND-associated antigen gene from genomic DNA, DNA fragments are generated to form a genomic library. Since some of the sequences encoding related ND-associated antigens are available and can be purified and labeled, the cloned DNA fragments in the genomic DNA library may be screened by nucleic acid hybridization to a labeled probe (Benton and Davis, 1977, Science 196: 180; Grunstein and Hogness, 1975, Proc. Natl. Acad. Sci. U.S.A. 72: 3961). Those DNA fragments with substantial homology to the probe will hybridize. It is also possible to identify an appropriate fragment by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map.

Alternatives to isolating the ND-associated antigen genomic DNA include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or synthesizing a cDNA to the mRNA which encodes the ND-associated antigen. For example, RNA for cDNA cloning of the ND-associated antigen gene can be isolated from cells which express the ND-associated antigen. A cDNA library may be generated by methods known in the art and screened by methods, such as those disclosed for screening a genomic DNA library. If an antibody to the ND-associated antigen is available, the ND-associated antigen may be identified by binding of a labeled antibody to the ND-associated antigen synthesizing clones.

Other specific embodiments for the cloning of a nucleotide sequence encoding an ND-associated antigen, are presented as examples but not by way of limitation, as follows: In a specific embodiment, nucleotide sequences encoding an ND-associated antigen protein can be identified and obtained by hybridization with a probe comprising a nucleotide sequence encoding ND-associated antigen under conditions of low to medium stringency. By way of example and not limitation, procedures using such conditions of low stringency are as follows (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. U.S.A. 78: 6789-6792). Filters containing DNA are pretreated for 6 h at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10^6 cpm ^{32}P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 h at 40°C, and then washed for 1.5 h at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 h at 60°C. Filters are blotted dry and

exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations).

Any technique for mutagenesis known in the art can be used to modify individual nucleotides in a DNA sequence, for purpose of making amino acid substitution(s) in the expressed peptide sequence, or for creating/deleting restriction sites to facilitate further manipulations. Such techniques include but are not limited to, chemical mutagenesis, *in vitro* site-directed mutagenesis (Hutchinson *et al.*, 1978, J. Biol. Chem. 253: 6551), oligonucleotide-directed mutagenesis (Smith, 1985, Ann. Rev. Genet. 19: 423-463; Hill *et al.*, 1987, Methods Enzymol. 155: 558-568), PCR-based overlap extension (Ho *et al.*, 1989, Gene 77: 51-59), PCR-based megaprimer mutagenesis (Sarkar *et al.*, 1990, Biotechniques 8: 404-407), *etc.* Modifications can be confirmed by double stranded dideoxynucleotide DNA sequencing.

4.1.4.1 Host–Vector Systems

Nucleotide sequences encoding an ND-associated antigenic polypeptide can be inserted into the expression vector for propagation and expression in recombinant cells. An expression construct, as used herein, refers to a nucleotide sequence encoding an ND-associated antigenic polypeptide operably associated with one or more regulatory regions which allows expression of the ND-associated antigenic polypeptide in an appropriate host cell. "Operably-associated" refers to an association in which the regulatory regions and the ND-associated antigenic polypeptide sequence to be expressed are joined and positioned in such a way as to permit transcription, and ultimately, translation of the ND-associated peptide or polypeptide sequence. A variety of expression vectors may be used for the expression of ND-associated antigenic polypeptides, including, but not limited to, plasmids, cosmids, phage, phagemids, or modified viruses. Examples include bacteriophages such as lambda derivatives, or plasmids such as pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene). Typically, such expression vectors comprise a functional origin of replication for propagation of the vector in an appropriate host cell, one or more restriction endonuclease sites for insertion of the ND-associated antigenic peptide gene sequence, and one or more selection markers.

Vectors based on *E. coli* are the most popular and versatile systems for high level expression of foreign proteins (Makrides, 1996, Microbiol. Rev. 60: 512-538). Non-limiting examples of prokaryotic expression vectors may include the λgt vector series such as λgt11 (Huynh *et al.*, 1984 in "DNA Cloning Techniques", Vol. I: A Practical Approach (D. Glover, ed.), pp. 49-78, IRL Press, Oxford), and the pET vector series (Studier *et al.*,

1990, Methods Enzymol., 185: 60-89). Non-limiting examples of regulatory regions that can be used for expression in *E. coli* may include but not limited to *lac*, *trp*, *lpp*, *phoA*, *recA*, *tac*, $\lambda P_{L,}$ and phage T3 and T7 promoters (Makrides, 1996, *supra*).

However, a potential drawback of a prokaryotic host-vector system is the inability to perform many of the post-translational processing events of mammalian cells. Thus, a eukaryotic host-vector system is preferred, a mammalian host-vector system is more preferred, and a human host-vector system is the most preferred. The regulatory regions necessary for transcription of the ND-associated antigenic peptide or polypeptide can be provided by the expression vector. A translation initiation codon (ATG) may also be provided to express a nucleotide sequence encoding an ND-associated antigenic polypeptide that lacks an initiation codon. In a compatible host-construct system, cellular proteins required for transcription, such as RNA polymerase and transcription factors, will bind to the regulatory regions on the expression construct to effect transcription of the ND-associated antigenic polypeptide sequence in the host organism. The precise nature of the regulatory regions needed for gene expression may vary from host cell to host cell. Generally, a promoter is required which is capable of binding RNA polymerase to initiate the transcription of an operably-associated nucleic acid sequence. Such regulatory regions may include those 5'-non-coding sequences involved with initiation of transcription and translation, such as a TATA box, cap site, a CAAT box, transcription factor binding sites, enhancer elements, and the like. The non-coding region 3' to the coding sequence may contain transcriptional termination regulatory sequences, such as terminators and polyadenylation sites.

Both constitutive and inducible regulatory regions may be used for expression of the ND-associated antigenic peptide or polypeptide. It may be desirable to use inducible promoters when the conditions optimal for growth of the recombinant cells and the conditions for high level expression of the ND-associated antigenic polypeptide are different. Examples of useful regulatory regions are provided in the next section below.

For expression of ND-associated antigenic polypeptides in mammalian host cells, a variety of regulatory regions can be used, for example, the SV40 early and late promoters, the cytomegalovirus (CMV) immediate early promoter, and the Rous sarcoma virus long terminal repeat (RSV-LTR) promoter. Inducible promoters that may be useful in mammalian cells include but are not limited to those associated with the metallothionein II gene, mouse mammary tumor virus glucocorticoid responsive long terminal repeats (MMTV-LTR), the β-interferon gene, and the Hsp70 gene (Williams *et al.*, 1989, Cancer Res. 49: 2735-42 ; Taylor *et al.*, 1990, Mol. Cell. Biol. 10: 165-75).

The following animal regulatory regions, which exhibit tissue specificity and have been utilized in transgenic animals, can also be used in cells of a particular tissue type of interest: elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, 1984, Cell 38: 639-646; Ornitz *et al.*, 1986, Cold Spring Harbor Symp. Quant. Biol. 50: 399-409; MacDonald, 1987, Hepatology 7: 425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315: 115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, 1984, Cell 38: 647-658; Adames *et al.*, 1985, Nature 318: 533-538; Alexander *et al.*, 1987, Mol. Cell. Biol. 7: 1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, 1986, Cell 45: 485-495), albumin gene control region which is active in liver (Pinkert *et al.*, 1987, Genes Dev. 1: 268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf *et al.*, 1985, Mol. Cell. Biol. 5: 1639-1648; Hammer *et al.*, 1987, Science 235: 53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey *et al.*, 1987, Genes Dev. 1: 161-171), beta-globin gene control region which is active in myeloid cells (Mogram *et al.*, 1985, Nature 315: 338-340; Kollias *et al.*, 1986, Cell 46: 89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead *et al.*, 1987, Cell 48: 703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314: 283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.*, 1986, Science 234: 1372-1378).

The efficiency of expression of the ND-associated antigenic peptide or polypeptide in a host cell may be enhanced by the inclusion of appropriate transcription enhancer elements in the expression vector, such as those found in SV40 virus, Hepatitis B virus, cytomegalovirus, immunoglobulin genes, metallothionein, β-actin (see Bittner *et al.*, 1987, Methods in Enzymol. 153: 516-544; Gorman, 1990, Curr. Op. in Biotechnol. 1: 36-47).

The expression vector may also contain sequences that permit maintenance and replication of the vector in more than one type of host cell, or integration of the vector into the host chromosome. Such sequences may include but are not limited to replication origins, autonomously replicating sequences (ARS), centromere DNA, and telomere DNA. It may also be advantageous to use shuttle vectors that can be replicated and maintained in at least two types of host cells.

In addition, the expression vector may contain selectable or screenable marker genes for initially isolating or identifying host cells that contain DNA encoding an ND-associated antigenic peptide. For long term, high yield production of ND-associated antigenic peptides, stable expression in mammalian cells is preferred. A number of

selection systems may be used for mammalian cells, including, but not limited, to the Herpes simplex virus thymidine kinase (Wigler *et al.*, 1977, Cell 11: 223), hypoxanthine-guanine phosphoribosyltransferase (Szybalski and Szybalski, 1962, Proc. Natl. Acad. Sci. U.S.A. 48: 2026), and adenine phosphoribosyltransferase (Lowy *et al.*, 1980, Cell 22: 817) genes can be employed in *tk*$^-$, *hgprt*$^-$ or *aprt*$^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dihydrofolate reductase (*dhfr*), which confers resistance to methotrexate (Wigler *et al.*, 1980, Natl. Acad. Sci. U.S.A. 77: 3567; O'Hare *et al.*, 1981, Proc. Natl. Acad. Sci. U.S.A. 78: 1527); gpt, which confers resistance to mycophenolic acid (Mulligan and Berg, 1981, Proc. Natl. Acad. Sci. U.S.A. 78: 2072); neomycin phosphotransferase (neo), which confers resistance to the aminoglycoside G-418 (Colberre-Garapin *et al.*, 1981, J. Mol. Biol. 150: 1); and hygromycin phosphotransferase (hyg), which confers resistance to hygromycin (Santerre *et al.*, 1984, Gene 30: 147). Other selectable markers, such as but not limited to histidinol and Zeocin™ can also be used.

In order to insert the DNA sequence of the ND-associated antigenic polypeptide into the cloning site of a vector, DNA sequences with regulatory functions, such as promoters, must be attached to DNA sequences encoding the ND-associated polypeptide. To do this, linkers or adapters providing the appropriate compatible restriction sites may be ligated to the ends of cDNA or synthetic DNA encoding an ND-associated antigenic polypeptide, by techniques well known in the art (Wu *et al.*, 1987, Methods Enzymol. 152: 343-349). Cleavage with a restriction enzyme can be followed by modification to create blunt ends by digesting back or filling in single-stranded DNA termini before ligation. Alternatively, a desired restriction enzyme site can be introduced into a fragment of DNA by amplification of the DNA by use of PCR with primers containing the desired restriction enzyme site.

The expression construct comprising an ND-associated antigenic polypeptide sequence operably associated with regulatory regions can be directly introduced into appropriate host cells for expression and production of ND-associated antigenic peptide without further cloning (see, for example, U.S. Patent No. 5,580,859). The expression constructs may also contain DNA sequences that facilitate integration of the ND-associated antigenic polypeptide sequence into the genome of the host cell, *e.g.*, via homologous recombination. In this instance, it is not necessary to employ an expression vector comprising a replication origin suitable for appropriate host cells in order to propagate and express the ND-associated antigenic peptide in the host cells.

Expression constructs containing cloned nucleotide sequence encoding ND-associated antigenic polypeptides can be introduced into the host cell by a variety of techniques known in the art, including but not limited to, for prokaryotic cells, bacterial

transformation (Hanahan, 1985, in DNA Cloning, A Practical Approach, 1: 109-136), and for eukaryotic cells, calcium phosphate mediated transfection (Wigler *et al.*, 1977, Cell 11: 223-232), liposome-mediated transfection (Schaefer-Ridder *et al.*, 1982, Science 215: 166-168), electroporation (Wolff *et al.*, 1987, Proc. Natl. Acad. Sci. 84: 3344), and microinjection (Cappechi, 1980, Cell 22: 479-488). Co-expression of an ND-associated antigenic peptide and a carrier protein in the same host cell can be achieved by essentially the same methods.

For long term, high yield production of properly processed ND-associated antigenic polypeptides or ND-associated antigenic peptide–carrier protein complexes, stable expression in mammalian cells is preferred. Cell lines that stably express ND-associated antigenic polypeptides may be engineered by using a vector that contains a selectable marker. By way of example but not limitation, following the introduction of the expression constructs, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the expression construct confers resistance to the selection and optimally allows cells to stably integrate the expression construct into their chromosomes and to grow in culture and to be expanded into cell lines. Such cells can be cultured for a long period of time while ND-associated antigenic polypeptide is expressed continuously.

Any of the cloning and expression vectors described herein may be synthesized and assembled from known DNA sequences by techniques well known in the art. The regulatory regions and enhancer elements can be of a variety of origins, both natural and synthetic. Some vectors and host cells may be obtained commercially. Non-limiting examples of useful vectors are described in Appendix 5 of Current Protocols in Molecular Biology, Ausubel *et al.* (eds.), Greene Publish. Assoc. & Wiley Interscience, New York, 1988, which is incorporated herein by reference; and the catalogs of commercial suppliers such as Clontech Laboratories, Stratagene Inc., and Invitrogen, Inc.

Alternatively, a number of viral-based expression systems may also be utilized with mammalian cells to produce ND-associated antigens. Vectors using DNA virus backbones have been derived from simian virus 40 (SV40) (Hamer *et al.*, 1979, Cell 17: 725), adenovirus (Van Doren *et al.*, 1984, Mol. Cell Biol. 4: 1653), adeno-associated virus (McLaughlin *et al.*, 1988, J. Virol. 62: 1963), and bovine papillomas virus (Zinn *et al.*, 1982, Proc. Natl. Acad. Sci. 79: 4897). In cases where an adenovirus is used as an expression vector, the donor DNA sequence may be ligated to an adenovirus transcription/translation control region, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or

E3) will result in a recombinant virus that is viable and capable of expressing heterologous products in infected hosts (see, *e.g.*, Logan and Shenk, 1984, Proc. Natl. Acad. Sci. U.S.A. 81: 3655-3659).

Bovine papillomavirus (BPV) can infect many higher vertebrates, including man, and its DNA replicates as an episome. A number of shuttle vectors have been developed for recombinant gene expression which exist as stable, multicopy (20-300 copies/cell) extrachromosomal elements in mammalian cells. Typically, these vectors contain a segment of BPV DNA (the entire genome or a 69% transforming fragment), a promoter with a broad host range, a polyadenylation signal, splice signals, a selectable marker, and "poisonless" plasmid sequences that allow the vector to be propagated in *E. coli*. Following construction and amplification in bacteria, the expression gene construct is transfected into cultured mammalian cells, for example, by the techniques of calcium phosphate coprecipitation or electroporation. For those host cells that do not manifest a transformed phenotype, selection of transformants is achieved by use of a dominant selectable marker, such as histidinol and G418 resistance. For example, BPV vectors such as pBCMGSNeo and pBCMGHis may be used to express ND-associated antigenic peptide sequences (Karasuyama *et al.*, Eur. J. Immunol. 18: 97-104; Ohe *et al.*, Human Gene Therapy 6: 325-33) which may then be transfected into a diverse range of cell types for expression of the ND-associated antigenic peptide.

Alternatively, the vaccinia 7.5K promoter may be used (see, e.g., Mackett *et al.*, 1982, Proc. Natl. Acad. Sci. U.S.A. 79: 7415-7419; Mackett *et al.*, 1984, J. Virol. 49: 857-864; Panicali *et al.*, 1982, Proc. Natl. Acad. Sci. U.S.A. 79: 4927-4931) In cases where a human host cell is used, vectors based on the Epstein-Barr virus (EBV) origin (OriP) and EBV nuclear antigen 1 (EBNA-1; a trans-acting replication factor) may be used. Such vectors can be used with a broad range of human host cells, *e.g.*, EBO-pCD (Spickofsky *et al.*, 1990, DNA Prot. Eng. Tech. 2: 14-18), pDR2 and λDR2 (available from Clontech Laboratories).

ND-associated antigenic peptides may also be made with a retrovirus-based expression system. In contrast to transfection, retroviruses can efficiently infect and transfer genes to a wide range of cell types including, for example, primary hematopoietic cells. In retroviruses such as Moloney murine leukemia virus, most of the viral gene sequences can be removed and replaced with nucleic acid sequences encoding the ND-associated antigen, while the missing viral functions can be supplied in *trans*. The host range for infection by a retroviral vector can also be manipulated by the choice of envelope used for vector packaging.

For example, a retroviral vector can comprise a 5' long terminal repeat (LTR), a 3' LTR, a packaging signal, a bacterial origin of replication, and a selectable marker. The ND-associated antigenic peptide DNA is inserted into a position between the 5' LTR and 3' LTR, such that transcription from the 5' LTR promoter transcribes the cloned DNA. The 5' LTR comprises a promoter, including but not limited to an LTR promoter, an R region, a U5 region and a primer binding site, in that order. Nucleotide sequences of these LTR elements are well known in the art. A heterologous promoter as well as multiple drug selection markers may also be included in the expression vector to facilitate selection of infected cells (see McLauchlin *et al.*, 1990, Prog. Nucleic Acid Res. and Molec. Biol. 38: 91-135; Morgenstern *et al.*, 1990, Nucleic Acid Res. 18: 3587-3596; Choulika *et al.*, 1996, J. Virol 70: 1792-1798; Boesen *et al.*, 1994, Biotherapy 6: 291-302; Salmons and Gunzberg, 1993, Human Gene Therapy 4: 129-141; and Grossman and Wilson, 1993, Curr. Opin. in Genetics and Devel. 3: 110-114).

Other useful eukaryotic host-vector system may include yeast and insect systems. In yeast, a number of vectors containing constitutive or inducible promoters may be used with *Saccharomyces cerevisiae* (baker's yeast), *Schizosaccharomyces pombe* (fission yeast), *Pichia pastoris,* and *Hansenula polymorpha* (methylotropic yeasts). For a review see, Current Protocols in Molecular Biology, Vol. 2, 1988, Ed. S *et al.*, Greene Publish. Assoc. & Wiley Interscience, Ch. 13; Grant *et al.*, 1987, Expression and Secretion Vectors for Yeast, in Methods in Enzymology, Eds. Wu & Grossman, 1987, Acad. Press, N.Y., Vol. 153, pp. 516-544; Glover, 1986, DNA Cloning, Vol. II, IRL Press, Wash., D.C., Ch. 3; and Bitter, 1987, Heterologous Gene Expression in Yeast, Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y., Vol. 152, pp. 673-684; and Vols. I and II of The Molecular Biology of the Yeast *Saccharomyces*, Strathern *et al.* (eds.), Cold Spring Harbor Press, Cold Spring Harbor 1982.

In an insect system a baculovirus, *Autographa californica* nuclear polyhidrosis virus (AcNPV), can be used as a vector to express an ND-associated antigenic peptide in *Spodoptera frugiperda* cells. The ND-associated antigenic peptide DNA may be cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). These recombinant viruses are then used to infect host cells in which the inserted DNA is expressed (see, *e.g.*, Smith *et al.*, 1983, J. Virol. 46: 584; Smith, U.S. Patent No. 4,215,051).

The expression vector must be used with a compatible host cell which may be derived from a prokaryotic or an eukaryotic organism, including, but not limited to bacteria, yeasts, insects, mammals, and humans. Any cell type that can produce ND-

associated antigenic polypeptides and is compatible with the expression vector may be used, including those that have been cultured *in vitro* or genetically engineered. Host cells may be obtained from normal or affected subjects, including healthy humans, patients with neurodegenerative disorders or disease, private laboratory deposits, public culture collections such as the American Type Culture Collection, or from commercial suppliers.

Preferred mammalian host cells include but are not limited to those derived from humans, monkeys and rodents, (see, for example, Kriegler M. in *Gene Transfer and Expression: A Laboratory Manual*, Freeman & Co., New York 1990), such as monkey kidney cell line transformed by SV40 (COS-7, ATCC CRL 1651), human embryonic kidney line (293, 293-EBNA), or 293 cells subcloned for growth in suspension culture (Graham *et al.*, 1977, J. Gen. Virol. 36: 59), baby hamster kidney cells (BHK, ATCC CCL 10), chinese hamster ovary-cells-DHFR (CHO, Urlaub and Chasin. Proc. Natl. Acad. Sci. 77; 4216, 1980), mouse sertoli cells (Mather, 1980, Biol. Reprod. 23: 243-251), mouse fibroblast cells (NIH-3T3), monkey kidney cells (CVI ATCC CCL 70), african green monkey kidney cells (VERO-76, ATCC CRL-1587), human cervical carcinoma cells (HELA, ATCC CCL 2), canine kidney cells (MDCK, ATCC CCL 34), buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75), human liver cells (Hep G2, HB 8065), and mouse mammary tumor cells (MMT 060562, ATCC CCL51). Exemplary cancer cell types used for demonstrating the utility of recombinant cells (producing ND-associated antigenic peptide-peptide complexes) as a cancer vaccine are provided as follows: mouse fibroblast cell line, NIH3T3, mouse Lewis lung carcinoma cell line, LLC, mouse mastocytoma cell line, P815, mouse lymphoma cell line, EL4 and its ovalbumin transfectant, E.G7, mouse melanoma cell line, B16F10, mouse fibrosarcoma cell line, MC57, and human small cell lung carcinoma cell lines, SCLC#2 and SCLC#7.

The recombinant cells may be cultured under standard conditions of temperature, incubation time, optical density, and media composition. Alternatively, a cells may be cultured under conditions emulating the nutritional and physiological requirements of a cell in which the ND-associated antigen is endogenously expressed.

The ND-associated antigenic protein, or an antigenic portion thereof, can be purified by any methods appropriate for the protein. Alternatively, as described below, such recombinant cells may be used to co-express ND-associated antigens together with recombinant carrier proteins that can be used for formation of ND-associated antigen vaccines. However, conditions for growth of recombinant cells may be different from those for expression of ND-associated antigenic polypeptides and carrier proteins. Modified culture conditions and media may be used to enhance production of carrier protein–ND-associated antigen complexes. For example, recombinant cells containing ND-

associated antigenic polypeptides with their cognate promoters may be exposed to heat or other environmental stress, or chemical stress. Any technique known in the art may be applied to establish the optimal conditions for producing ND-associated antigenic polypeptides.

4.1.4.2 Purification Methods for Recombinant ND-Associated Antigens

Generally, the recombinant ND-associated antigenic peptides and polypeptides of the invention can be recovered and purified from recombinant cell cultures by known methods, including ammonium sulfate precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, immunoaffinity chromatography, hydroxyapatite chromatography, and lectin chromatography.

In one embodiment, the invention provides methods for purification of recombinant ND-associated antigenic peptides and polypeptides by affinity purification. The principle of affinity chromatography well known in the art. One approach is based on specific molecular interactions between an affinity label present on the ND-associated antigenic polypeptide and its binding partner. A second approach, immunoaffinity chromatography, relies on the immunospecific binding of an antibody to an epitope present on the tag.

Described below are several methods based on specific molecular interactions of a tag and its binding partner. Protein A affinity chromatography, a method that is generally applicable to purifying recombinant ND-associated antigens that are fused to the constant regions of immunoglobulin, is a well known technique in the art. Staphylococcus protein A is a 42 kD polypeptide that binds specifically to a region located between the second and third constant regions of heavy chain immunoglobulins. Because of the Fc domains of different classes, subclasses and species of immunoglobulins, affinity of protein A for human Fc regions is strong, but may vary with other species. Subclasses that are less preferred include human IgG-3, and most rat subclasses. For certain subclasses, protein G (of Streptococci) may be used in place of protein A in the purification. Protein-A sepharose (Pharmacia or Biorad) is a commonly used solid phase for affinity purification of antibodies, and can be used essentially in the same manner for the purification of ND-associated antigenic polypeptide fused to an immunoglobulin Fc fragment. ND-associated antigenic polypeptide present in cell lysate or, if secreted by the cell, in the supernatant, binds specifically to protein A on the solid phase, while the contaminants are washed away. Bound ND-associated antigenic polypeptide can be eluted by various buffer systems known in the art, including a succession of citrate, acetate and glycine-HCl buffers which gradually lowers the pH. This method is less preferred if the

recombinant cells also produce antibodies which will be copurified with the ND-associated antigenic polypeptide. See, for example, Langone, 1982, J. Immunol. Meth. 51: 3; Wilchek *et al.*, 1982, Biochem. Intl. 4: 629; Sjobring *et al.*, 1991, J. Biol. Chem. 26: 399; page 617-618, in Antibodies A Laboratory Manual, edited by Harlow and Lane, Cold Spring Harbor laboratory, 1988.

Alternatively, a polyhistidine tag may be used, in which case, the ND-associated antigenic polypeptide can be purified by metal chelate chromatography. The polyhistidine tag, usually a sequence of six histidines, has a high affinity for divalent metal ions, such as nickel ions (Ni^{2+}), which can be immobilized on a solid phase, such as nitrilotriacetic acid matrices. Polyhistidine has a well characterized affinity for Ni^{2+}-NTA-agarose, and can be eluted with either of two mild treatments: imidazole (0.1-0.2 M) will effectively compete with the resin for binding sites; or lowering the pH just below 6.0 will protonate the histidine side-chains and disrupt the binding. The purification method comprises loading the cell culture supernatant onto the Ni^{2+}-NTA-agarose column, washing the contaminants through, and eluting the ND-associated antigenic polypeptide with imidazole or weak acid. Ni^{2+}-NTA-agarose can be obtained from commercial suppliers such as Sigma (St. Louis) and Qiagen. Antibodies that recognize the polyhistidine tag are also available which can be used to detect and quantify the ND-associated antigenic polypeptide.

Another exemplary affinity label that can be used is the glutathione-S-transferase (GST) sequence, originally cloned from the helminth, *Schistosoma japonicum*. In general, an ND-associated antigenic polypeptide–GST fusion expressed in a prokaryotic host cell, such as *E. coli*, can be purified from the cell culture supernatant by absorption with glutathione agarose beads, followed by elution in the presence of free reduced glutathione at neutral pH. Denaturing conditions are not required at any stage during purification. Moreover, since GST is known to form dimers under certain conditions, dimeric ND-associated antigenic peptides may be obtained. See, Smith, 1993, Methods Mol. Cell Bio. 4: 220-229.

Another useful affinity label that can be used is the maltose binding protein (MBP) of *E. coli*, which is encoded by the *mal*E gene. The secreted ND-associated polypeptide-MBP present in the cell supernatant binds to amylose resin while contaminants are washed away. The bound ND-associated polypeptide-MBP is eluted from the amylose resin by maltose. See, for example, Guan *et al.*, 1987, Gene 67: 21-30.

The second approach for purifying ND-associated antigenic peptides is applicable to affinity labels that contain an epitope for which polyclonal or monoclonal antibodies are available. Various methods known in the art for purification of protein by

immunospecific binding, such as immunoaffinity chromatography, and immunoprecipitation, can be used. See, for example, Chapter 13 in Antibodies A Laboratory Manual, edited by Harlow and Lane, Cold Spring Harbor laboratory, 1988; and Chapter 8, Sections I and II, in Current Protocols in Immunology, ed. by Coligan *et al.*, John Wiley, 1991; the disclosure of which are both incorporated by reference herein.

The embodiments described above may also be used to recover and purify ND-associated antigenic peptide complexes from the cell culture medium of mammalian cells, such as human cells expressing an ND-associated peptides of the invention. The methods can be adapted to perform medium and large scale purification of an ND-associated antigenic peptide. Methods that do not require lowering pH or denaturing conditions are most preferred for purification of ND-antigenic peptides. The methods may be used to isolate ND-associated antigenic peptides from eukaryotic cells, for example, cells of neuronal origin or cells isolated from a subject with a neurodegenerative disorder.

4.2 Therapeutic Uses of ND-Associated Antigen Vaccines

The present invention encompasses the use of vaccines in methods for treatment of and prevention of neurodegenerative disorders. In various embodiments described in detail herein, an effective amount of an ND-associated antigenic molecule, together with one or more pharmaceutically acceptable carriers, is administered to a patient for therapeutic purposes.

4.2.1 Prevention and Treatment of Neurodegenerative Disorders

For prevention and treatment of neurodegenerative disorders, an ND-associated antigen that displays the antigenicity of an antigen of an neurodegenerative disease or disorder is prepared, and used as a vaccine against the disease or disorder. As will be appreciated by those skilled in the art, the antigens may isolated from any cell that displays the antigenicity of an antigen associated with the neurodegenerative disorder. For example, cells may express the ND-associated antigen itself, or alternatively, cells may be infected by or engineered to express a fragment or non-pathogenic form of the ND-associated antigen.

Neurodegenerative disorders include disorders relating to the central nervous system and/or peripheral nervous system including, but not limited to, cognitive and neurodegenerative disorders such as Alzheimer's Disease, age-related loss of cognitive function and senile dementia, Parkinson's disease, amyotrophic lateral sclerosis, Wilson's Disease, cerebral and progressive supranuclear palsy, Guam disease, Lewy body dementia, prion diseases, such as spongiform encephalopathies, *e.g.*, Creutzfeldt-Jakob disease,

polyglutamine diseases, such as Huntington's disease, myotonic dystrophy, Freidrich's ataxia and other ataxias, well as Gilles de la Tourette's syndrome, seizure disorders such as epilepsy and chronic seizure disorder, stroke, brain or spinal cord trauma, AIDS dementia, alcoholism, autism, retinal ischemia, glaucoma, autonomic function disorders such as hypertension and sleep disorders, and neuropsychiatric disorders that include, but are not limited to schizophrenia, schizoaffective disorder, attention deficit disorder, dysthymic disorder, major depressive disorder, mania, obsessive-compulsive disorder, psychoactive substance use disorders, anxiety, panic disorder, as well as unipolar and bipolar affective disorders. Additional neuropsychiatric and neurodegenerative disorders include, for example, those listed in the American Psychiatric Association's Diagnostic and Statistical manual of Mental Disorders (DSM), the most current version of which is incorporated herein by reference in its entirety.

In a preferred aspect of the invention, the purified ND-associated antigen vaccines may have particular utility in the treatment of human neurodegenerative disorders. It is appreciated, however, that the vaccines developed using the principles described herein will be useful in treating diseases of other mammals, for example, farm animals including: cattle; horses; sheep; goats; and pigs, and household pets including: cats; and dogs, that have similar pathologies.

4.2.2 Combination With Adoptive Immunotherapy

Adoptive immunotherapy refers to a therapeutic approach for treating neurodegenerative diseases in which immune cells are administered to a host with the aim that the cells mediate specific immunity, either directly or indirectly, to cells that express ND-associated antigens and/or antigenic components, and result in treatment of the neurodegenerative disorder, or prevention of the neurodegenerative disorder, as the case may be (see U.S. Patent Application Serial No. 08/527,546, filed September 13, 1995, which is incorporated by reference herein in its entirety).

According to the invention, therapy by administration of ND–associated antigen complexes, using any desired route of administration, is combined with adoptive immunotherapy using APC sensitized with ND–associated antigenic molecule complexes. The peptide complex-sensitized APC can be administered concurrently with the ND-associated antigen–peptide complexes, or before or after administration of the ND-associated antigen–peptide complexes. Furthermore, the mode of administration can be varied, including but not limited to, *e.g.*, subcutaneously, intravenously, intraperitoneally, intramuscularly, intradermally or mucosally.

4.2.2.1 Sensitization of Macrophages and Antigen Presenting Cells with ND-Associated Antigen Vaccines

The antigen-presenting cells, including but not limited to macrophages, dendritic cells and B-cells, are preferably obtained by production *in vitro* from stem and progenitor cells from human peripheral blood or bone marrow as described by Inaba *et al.*, 1992, J. Exp. Med. 176:1693-1702. APC can be obtained by any of various methods known in the art. In a preferred aspect human macrophages are used, obtained from human blood cells.

By way of example, but not limitation, macrophages can be obtained as follows: Mononuclear cells are isolated from peripheral blood of a patient (preferably the patient to be treated), by Ficoll-Hypaque gradient centrifugation and are seeded on tissue culture dishes which are pre-coated with the patient's own serum or with other AB+ human serum. The cells are incubated at 37°C for 1 hr, then non-adherent cells are removed by pipetting. To the adherent cells left in the dish, is added cold (4°C) 1 mM EDTA in phosphate-buffered saline and the dishes are left at room temperature for 15 minutes. The cells are harvested, washed with RPMI buffer and suspended in RPMI buffer. Increased numbers of macrophages may be obtained by incubating at 37°C with macrophage-colony stimulating factor (M-CSF); increased numbers of dendritic cells may be obtained by incubating with granulocyte-macrophage-colony stimulating factor (GM-CSF) as described in detail by Inaba *et al.*, 1992, J. Exp. Med. 176:1693-1702.

APC are sensitized with ND-associated antigenic molecules by incubating the cells *in vitro* with the complexes. The APC are sensitized with ND-associated antigens preferably by incubating *in vitro* with the antigen at 37°C for 15 minutes to 24 hours. By way of example but not limitation, $4x10^7$ macrophages can be incubated with 10-100 micrograms of ND-associated antigen per ml at 37°C for 15 minutes-24 hours in 1 ml plain RPMI medium. The cells are washed three times and resuspended in a physiological medium preferably sterile, at a convenient concentration (*e.g.*, $1x10^7$/ml) for injection in a patient. In a preferred embodiment, the antigen presenting cells are autologous to the patient, that is, the patient into which the sensitized APCs are injected is the patient from which the APC were originally isolated,.

Optionally, the ability of sensitized APC to stimulate, for example, the antigen-specific, class I-restricted cytotoxic T-lymphocytes (CTL) can be monitored by their ability to stimulate CTLs to release tumor necrosis factor, and by their ability to act as targets of such CTLs.

4.2.2.2 Reinfusion of Sensitized APC

The ND-antigen-sensitized APC are reinfused into the patient systemically, preferably intravenously, by conventional clinical procedures. These activated cells are reinfused, preferentially by systemic administration into the autologous patient. Patients generally receive from about 10^6 to about 10^{12} sensitized macrophages, depending on the condition of the patient. In some regimens, patients may optionally receive in addition a suitable dosage of a biological response modifier including but not limited to the cytokines IFN-α, IFN-γ, IL-2, IL-4, IL-6, TNF or other cytokine growth factor.

4.3 Determination of Immunogenicity of ND-Associated Antigen Vaccines

Optionally, the ND-associated antigen vaccines can be assayed for immunogenicity using any method known in the art. By way of example but not limitation, one of the following three procedures can be used.

4.3.1 MLTC Assay

Briefly, mice are injected with the hsp ND-associated antigen, using any convenient route of administration. As a negative control, other mice are injected with cells not associated with ND-associated antigens. Cells containing ND-associated antigens may act as a positive control for the assay. The mice are injected twice, 7-10 days apart. Ten days after the last immunization, the spleens are removed and the lymphocytes released. The released lymphocytes may be re-stimulated subsequently *in vitro* by the addition of dead cells that expressed the antigen of interest.

For example, $8x10^6$ immune spleen cells may be stimulated with $4x10^4$ mitomycin C treated or γ-irradiated (5-10,000 rads) cells containing the antigen of interest (or cells transfected with an appropriate gene, as the case may be) in 3ml RPMI medium containing 10% fetal calf serum. In certain cases 33% secondary mixed lymphocyte culture supernatant may be included in the culture medium as a source of T cell growth factors (*See,* Glasebrook, et al., 1980, *J. Exp. Med.* 151:876). To test the primary cytotoxic T cell response after immunization, spleen cells may be cultured without stimulation. In some experiments spleen cells of the immunized mice may also be re-stimulated with antigenically distinct cells, to determine the specificity of the cytotoxic T cell response.

Six days later the cultures are tested for cytotoxicity in a 4 hour ^{51}Cr-release assay (*see* Palladino *et al.*, 1987, *Cancer Res.* 47:5074-5079 and Blachere, at al., 1993, *J. Immunotherapy* 14:352-356). In this assay, the mixed lymphocyte culture is added to a target cell suspension to give different effector:target (E:T) ratios (usually 1:1 to 40:1). The

target cells are prelabelled by incubating $1x10^6$ target cells in culture medium containing 20 mCi ^{51}Cr/ml for one hour at 37°C. The cells are washed three times following labeling. Each assay point (E:T ratio) is performed in triplicate and the appropriate controls incorporated to measure spontaneous ^{51}Cr release (no lymphocytes added to assay) and 100% release (cells lysed with detergent). After incubating the cell mixtures for 4 hours, the cells are pelletted by centrifugation at 200g for 5 minutes. The amount of ^{51}Cr released into the supernatant is measured by a gamma counter. The percent cytotoxicity is measured as cpm in the test sample minus spontaneously released cpm divided by the total detergent released cpm minus spontaneously released cpm.

In order to block the MHC class I cascade a concentrated hybridoma supernatant derived from K-44 hybridoma cells (an anti-MHC class I hybridoma) is added to the test samples to a final concentration of 12.5%.

Primary T cells are obtained from spleen, fresh blood, or CSF and purified by centrifugation using FICOLL-PAQUE PLUS (Pharmacia, Upsalla, Sweden) essentially as described by Kruse and Sebald, 1992, EMBO J. 11: 3237-3244. The peripheral blood mononuclear cells are incubated for 7-10 days with a lysate of cells expressing an ND-associated antigen. Antigen presenting cells may, optionally be added to the culture 24 to 48 hours prior to the assay, in order to process and present the antigen in the lysate. The cells are then harvested by centrifugation, and washed in RPMI 1640 media (GibcoBRL, Gaithersburg, Md.). $5x10^4$ activated T cells/well (PHA-blasts) are in RPMI 1640 media containing 10% fetal bovine serum, 10 mM HEPES, pH 7.5, 2 mM L-glutamine, 100 units/ml penicillin G, and 100 µg/ml streptomycin sulphate in 96 well plates for 72 hrs at 37°C., pulsed with 1 µCi ^3H-thymidine (DuPont NEN, Boston, Mass.)/well for 6 hrs, harvested, and radioactivity measured in a TOPCOUNT scintillation counter (Packard Instrument Co., Meriden, Conn.).

4.3.2 Antibody Response Assay

In one embodiment of the invention, the immunogenicity of an ND-associated antigen or ND-associated antigen adjuvant complex is determined by measuring antibodies produced in response to the vaccination with the antigen or complex, by an antibody response assay, such as an enzyme-linked immunosorbent assay (ELISA) assay. Methods for such assays are well known in the art (see, *e.g.*, Section 2.1 of Current Protocols in Immunology, Coligan *et al.* (eds.), John Wiley and Sons, Inc. 1997). In one mode of the embodiment, microtitre plates (96-well Immuno Plate II, Nunc) are coated with 50 µl/well of a 0.75 µg/ml solution of a purified, non-adjuvant-complexed form of the ND-

associated antigen used in the vaccine (*e.g.* tau protein) in PBS at 4°C for 16 hours and at 20°C for 1 hour. The wells are emptied and blocked with 200 µl PBS-T-BSA (PBS containing 0.05% (v/v) TWEEN 20 and 1% (w/v) bovine serum albumin) per well at 20°C for 1 hour, then washed 3 times with PBS-T. Fifty µl/well of plasma or CSF from a vaccinated animal (such as a model mouse or a human patient) is applied at 20°C for 1 hour, and the plates are washed 3 times with PBS-T. The anti-ND associated antigen antibody activity is then measured calorimetrically after incubating at 20°C for 1 hour with 50µl/well of sheep anti-mouse or anti-human immunoglobulin, as appropriate, conjugated with horseradish peroxidase (Amersham) diluted 1:1,500 in PBS-T-BSA and (after 3 further PBS-T washes as above) with 50 µl of an o-phenylene diamine (OPD)-H_2O_2 substrate solution. The reaction is stopped with 150 µl of 2M H_2SO_4 after 5 minutes and absorbance is determined in a Kontron SLT-210 photometer (SLT Lab-instr., Zurich, Switzerland) at 492 nm (ref. 620 nm).

4.4 Methods for Diagnosing and Assaying Progress of Neurodegenerative Disorders, Such as AD

There are presently many molecular markers known in the art for the diagnosis of neurodegenerative disorders, such as AD (see, *e.g.*, Bancher et al., 1998, J. Neural Transm., Suppl. 53:185-197; Galasko, 1998, J. Neural Transm., Suppl. 53:209-221; issue no. 2 of Neurobiol. Aging, Vol. 19, 1998, including articles by Arai et al., pp. 125-6; Foster, pp. 127-129; Mayeux, pp.139-143; Klunk, pp. 145-157; Hock, pp. 149-151; Robles, pp. 153-157; Hyman, pp. 159-160; and Lannfelt, pp. 165-167). These methods can be used to determine whether an asymptomatic human subject displays any of the molecular hallmarks of a specific neurodegenerative disorder. The methods may also be used to diagnose a neurodegenerative disorder in a human subject who exhibits symptoms of the early stages of the neurodegenerative disorder. These methods are useful for identifying individuals at risk of a neurodegenerative disorder who would benefit from the methods of the invention of treatment and prevention of neurodegenerative disorders. Finally, the methods can also be used to assay the efficacy of the vaccines of the present invention and monitor the progress of a neurodegenerative disorder in those receiving the vaccines. The diagnostic methods to be utilized according to the present invention include but are not limited to testing for molecular indicators of a particular neurodegenerative disorder, such as AD, or for alterations in neurophysiological function that would be affected by such a neurodegenerative disorder. In a preferred embodiment, more than one of the assays described below would be carried out to confirm the diagnosis of the presence or the extent of progession of the neurodegenerative disorder.

4.4.1 Molecular Indicators of Neurodegenerative Disorders

Testing for the presence of phosphorylated tau protein using a monoclonal antibody specific for the phosphorylated form, as disclosed in U.S. Patent No. 5,733,734, serves as an indicator for neurodegenerative disorders, such as AD. In a preferred embodiment, the monoclonal antibody is Alz-50 (U.S. Patent No. 5,811,310). The presence of phosphorylated Tau may be tested in either brain tissue or cerebrospinal fluid or cultures of olfactory neurons from the patient.

Tau proteolysis products have been found to be present in the blood or spinal fluids of individuals with AD (U.S. Patent No. 5,492,812). Thus, testing for the presence of tau peptides in blood or spinal fluid samples may provide a diagnostic measure of the presence or progression of AD in patients and other individuals.

The presence or extent of AD can also be determined by measuring the relative abundance of Aβ42 and Aβ40. In normal individuals, the amount of Aβ40 far exceeds the amount of Aβ42. In contrast, Aβ42 predominates in AD patients. Additionally, all mutations implicated in FAD, whether in the *APP*, *PS1*, or *PS2* genes, relate to the processing of APP, and are thought to produce AD through promoting the synthesis of Aβ42. Thus, the relative amount of Aβ40 and Aβ42, for example in a CSF sample or tissue biopsy from the brain or pancreas from an individual, would be an indicator of the presence or progress of AD in the individual.

Calcium activated neutral proteases are enzymes that regulate signal transduction by modulating the activities of signaling molecules (*e.g.* protein kinases and phosphatases) through partial proteolysis. Calcium activated neutral proteases are themselves regulated by partial proteolysis, wherein in the presence of calcium a precursor form of an enzyme undergoes autoproteolytic cleavage to produce a functional enzyme. It has been shown that the ratios of cleaved to uncleaved calcium activated neutral proteases are altered in AD patients. Thus, by measuring the relative amounts of each isoform in a test subject in comparison with a control subject, it is possible to detect AD in an individual (U.S. Patent No. 5,624,807).

It has been demonstrated that patients with neurodegenerative disorders, such as AD, have elevated levels of acetylcholinesterase (AChE) activity in ocular fluids. A colorimetric assay for the determination of AChE activity described by Ellman et al. (1961, Biochem. Pharmacol. 7:161-177) may be utilized to measure AchE activity levels in ocular fluid samples, *i.e.* aqueous humor or vitreous humor samples, the result of which would indicate the presence or absence and possibly the extent of AD (U.S. Patent No. 5,595,883).

One of the characteristics of neurodegenerative disorders, such as AD, is an impairment in cytokine secretion, for example IL-1, IL-3 and IL-6. It has been postulated

that the impairment is a downstream effect of impaired neural function. Thus it would be possible to assay for blood cytokine levels as indicators of AD (U.S. Patent No. 5,874,312)

Unless indicated otherwise, the proteins or peptides described *supra* may be assayed for by a radioimmunoassay, an enzyme-linked immunosorbant assay (ELISA), a sandwich assay, a gel immunodiffusion assay, an agglutination assay, a fluorescent immunoassay, a protein A immunoassay or an immunoelectrophoresis assay, or any other method known in the art. These methods are well known to those skilled in the art.

4.4.2 Neurophysiological Tests

U.S. Patent No.5,778,893 discloses methods of diagnosing AD, including the extent of AD, in an individual by applying agents that agonize or antagonize neuromuscular signaling and determining the response of the individual to said agents in comparison to control individuals. For example, a cholinergic antagonist is administered to the eye of an individual, the pupil allowed to dilate in response to the cholinergic antagonist, and the rate of which the pupil returns to its normal diameter measured and compared to the corresponding rates in control individuals.

In an alternative method of measuring neurophysiological output, a light source illuminates the eye of an individual suspected to have AD. The response of the individual's pupils to the light is measured by a computer system connected to a video camera that records the response of the pupils (U.S. Patent 5,883,691).

4.5 Adjuvants and Carrier Molecules

ND-associated antigens are administered with one or more adjuvants. In one embodiment, the ND-associated antigen is administered together with a mineral salt adjuvants or mineral salt gel adjuvant. Such mineral salt and mineral salt gel adjuvants include, but are not limited to, aluminum hydroxide (ALHYDROGEL, REHYDRAGEL), aluminum phosphate gel, aluminum hydroxyphosphate (ADJU-PHOS), and calcium phosphate.

In another embodiment, ND-associated antigen is administered with an immunostimulatory adjuvant. Such class of adjuvants, include, but are not limited to, cytokines (*e.g.*, interleukin-2, interleukin-7, interleukin-12, granulocyte-macrophage colony stimulating factor (GM-CSF), interferon-γ, interleukin-1β (IL-1β), and IL-1β peptide or Sclavo Peptide), cytokine-containing liposomes, triterpenoid glycosides or saponins (*e.g.*, QuilA and QS-21, also sold under the trademark STIMULON, ISCOPREP), Muramyl Dipeptide (MDP) derivatives, such as N-acetyl-muramyl-L-threonyl-D-isoglutamine (Threonyl-MDP, sold under the trademark TERMURTIDE), GMDP, N-acetyl-nor-

muramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine, muramyl tripeptide phosphatidylethanolamine (MTP-PE), unmethylated CpG dinucleotides and oligonucleotides, such as bacterial DNA and fragments thereof, LPS, monophosphoryl Lipid A (3D-MLAsold under the trademark MPL), and polyphosphazenes.

In another embodiment, the adjuvant used is a particulate adjuvant, including, but not limited to, emulsions, *e.g.*, Freund's Complete Adjuvant, Freund's Incomplete Adjuvant, squalene or squalane oil-in-water aduvant formulations, such as SAF and MF59, *e.g.*, prepared with block-copolymers, such as L-121 (polyoxypropylene/polyoxyethylene) sold under the trademark PLURONIC L-121, Liposomes, Virosomes, cochleates, and imune stimulating complex, which is sold under the trademark ISCOM.

In another embodiment, a microparticulate adjuvant is used. Microparticulate adjuvants include, but are not limited to biodegradable and biocompatible polyesters, homo- and copolymers of lactic acid (PLA) and glycolic acid (PGA), poly(lactide-*co*-glycolides) (PLGA) microparticles, polymers that self-associate into particulates (poloxamer particles), soluble polymers (polyphosphazenes), and virus-like particles (VLPs) such as recombinant protein particulates, *e.g.*, hepatiis B surface antigen (HbsAg).

Yet another class of adjuvants that may be used include mucosal adjuvants, including but not limited to heat-labile enterotoxin from *Escherichia coli* (LT), cholera holotoxin (CT) and cholera Toxin B Subunit (CTB) from *Vibrio cholerae*, mutant toxins (*e.g.* LTK63 and LTR72), microparticles, and polymerized liposomes.

In other embodiments, any of the above classes of adjuvants may be used in combination with each other or with other adjuvants. For example, non-limiting examples of combination adjuvant preparations that can be used to administer the ND-associated antigens of the invention include liposomes containing immunostimulatory protein, cytokines, or T-cell and/or B-cell peptides, or microbes with or without entrapped IL-2 or microparticles containing enterotoxin. Other adjuvants known in the art are also included within the scope of the invention (see *Vaccine Design: The Subunit and Adjuvant Approach*, Chap. 7, Michael F. Powell and Mark J. Newman (eds.), Plenum Press, New York, 1995, which is incorporated herein in its entirety).

The effectiveness of an adjuvant may be determined by measuring the induction of antibodies directed against an immunogenic polypeptide containing a HTLV env polypeptide epitope, the antibodies resulting from administration of this polypeptide in vaccines which are also comprised of the various adjuvants.

The polypeptides may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids, such as, for example, hydrochloric or phosphoric acids, or organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with free carboxyl groups may also be derived from inorganic bases, such as, for example, sodium potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

The vaccines of the invention may be multivalent or univalent. Multivalent vaccines are made from recombinant viruses that direct the expression of more than one antigen.

Many methods may be used to introduce the vaccine formulations of the invention; these include but are not limited to oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal routes, and via scarification (scratching through the top layers of skin, *e.g.*, using a bifurcated needle).

The patient to which the vaccine is administered is preferably a mammal, most preferably a human, but can also be a non-human animal including but not limited to cows, horses, sheep, pigs, fowl (*e.g.*, chickens), goats, cats, dogs, hamsters, mice and rats.

4.6 Dosage Regimens

Dosages of ND-associated antigens and antigen-adjuvant complexes can be extrapolated from prior art methods established in experimental tumor models (Blachere *et al.*, 1993, J. Immunotherapy 14:352-356). Extrapolation to human dosages of based on body weight and surface area. For example, prior art methods of extrapolating human dosage based on body weight can be carried out as follows: since the conversion factor for converting the mouse dosage to human dosage is Dose Human per kg = Dose Mouse per kg x 12 (*See* Freireich, E.J., *et al.*, 1966, Cancer Chemotherap. Rep. 50: 219-244), the effective dose of ND-associated antigen vaccines in humans weighing 70kg should be 1mg/kg ÷ 12 x 70, *i.e.*, about 6mg (5.8mg).

Drug doses are also given in milligrams per square meter of body surface area because this method rather than body weight achieves a good correlation to certain metabolic and excretionary functions (Shirkey, 1965, JAMA 193: 443). Moreover, body surface area can be used as a common denominator for drug dosage in adults and children as well as in different animal species as indicated below in Table 1 (Freireich *et al.*, 1966, Cancer Chemotherap. Rep. 50: 219-244).

TABLE 1

REPRESENTATIVE SURFACE AREA TO WEIGHT RATIOS (km) FOR VARIOUS SPECIES[1]

Species	Body Weight (kg)	Surface Area (Sqm)	km Factor
Mouse	0.02	0.0066	3.0
Rat	0.15	0.025	5.9
Monkey	3.0	0.24	12
Dog	8.0	0.40	20
Human, Child	20	0.80	25
Adult	60	1.6	37

Example: To express a mg/kg dose in any given species as the equivalent mg/sq m dose, multiply the dose by the appropriate km factor. In adult human, 100mg/kg is equivalent to 100 mg/kg x 37 kg/sq m = 3700 mg/sq m.

The doses recited above are preferably given once weekly for a period of about 4-6 weeks, and the mode or site of administration is preferably varied with each administration. In a preferred example, intradermal administrations are given, with each site of administration varied sequentially. Thus, by way of example and not limitation, the first injection may be given subcutaneously on the left arm, the second on the right arm, the third on the left belly, the fourth on the right belly, the fifth on the left thigh, the sixth on the right thigh, *etc.* The same site may be repeated after a gap of one or more injections. Also, split injections may be given. Thus, for example, half the dose may be given in one site and the other half on an other site on the same day.

Alternatively, the mode of administration is sequentially varied, *e.g.*, weekly injections are given in sequence intradermally, subcutaneously, intramuscularly, intravenously or intraperitoneally.

After 4-6 weeks, further injections are preferably given at two-week intervals over a period of time of one month. Later injections may be given monthly. The pace of later injections may be modified, depending upon the patient's clinical progress and responsiveness to the immunotherapy.

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[1] Freireich, *et al.*, 1966, Cancer Chemotherap. Rep. 50: 219-244.

4.7 Vaccine Formulation

ND-associated antigenic molecules purified by the methods of the invention may be formulated into pharmaceutical preparations for administration to mammals for treatment or prevention of neurodegenerative disorders and diseases. Drug solubility and the site of absorption are factors which should be considered when choosing the route of administration of a therapeutic agent. ND-associated antigenic molecules of the invention may be administered using any desired route of administration, including but not limited to, *e.g.*, subcutaneously, intravenously or intramuscularly, although intradermally is preferred. Advantages of intradermal administration include use of lower doses and rapid absorption, respectively. Mucosal routes of administration include, but are not limited to, oral, rectal and nasal administration. Preparations for mucosal administrations are suitable in various formulations as described below. The route of administration can be varied during a course of treatment.

Compositions comprising ND-associated antigens formulated in a compatible pharmaceutical carrier may be prepared, packaged, and labeled for treatment of the indicated neurodegenerative disorder. In preferred aspects, an amount of ND-associated peptide antigen vaccine is administered to a human that is in the range of about 2 to 150 μg, preferably 20 to 20 μg, most preferably about 5 μg, given once weekly for about 4-6 weeks, intradermally with the site of administration varied sequentially.

If the complex is water-soluble, then it may be formulated in an appropriate buffer, for example, phosphate buffered saline or other physiologically compatible solutions. Alternatively, if the resulting complex has poor solubility in aqueous solvents, then it may be formulated with a non-ionic surfactant such as Tween, or polyethylene glycol. Thus, the ND-associated peptide antigen vaccines and their physiologically acceptable solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral, rectal administration.

For oral administration, the pharmaceutical preparation may be in liquid form, for example, solutions, syrups or suspensions, or may be presented as a drug product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinized maize starch, polyvinyl

pyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well-known in the art.

Preparations for oral administration may be suitably formulated to give controlled release of the complexes. Such compositions may take the form of tablets or lozenges formulated in conventional manner.

In a specific embodiment, the ND-associated peptide antigen vaccines of the present invention are administered intrathecally by an implant be placed in or near the lesioned area of the nervous system. Suitable implants include, but are not limited to, gelfoam, wax, liposome or microparticle-based implants. Such compositions are preferably used when it is desired to achieve sustained release of the ND-associated peptide antigen vaccine.

For administration by inhalation, the complexes may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the complexes and a suitable powder base such as lactose or starch.

The complexes may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The complexes may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the complexes may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the complexes may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange

resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophilic drugs.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

The invention also provides kits for carrying out the therapeutic regimens of the invention. Such kits comprise in one or more containers therapeutically or prophylactically effective amounts of ND-associated peptide antigen in pharmaceutically acceptable form. The ND-associated peptide antigen in a vial of a kit of the invention may be in the form of a pharmaceutically acceptable solution, *e.g.*, in combination with sterile saline, dextrose solution, or buffered solution, or other pharmaceutically acceptable sterile fluid. Alternatively, the complex may be lyophilized or desiccated; in this instance, the kit optionally further comprises in a container a pharmaceutically acceptable solution (e.g., saline, dextrose solution, *etc.*), preferably sterile, to reconstitute the complex to form a solution for injection purposes.

In another embodiment, a kit of the invention further comprises a needle or syringe, preferably packaged in sterile form, for injecting the complex, and/or a packaged alcohol pad. Instructions are optionally included for administration of ND-associated peptide antigen vaccine by a clinician or by the patient.

4.7.1 Determination of Vaccine Efficacy

The immunopotency of ND-associated antigens can be determined by monitoring the immune response in test animals following immunization with the ND-associated antigen, or by use of any immunoassay known in the art. Generation of a humoral (antibody) response and/or cell-mediated immunity, may be taken as an indication of an immune response. Test animals may include mice, hamsters, dogs, cats, monkeys, rabbits, chimpanzees, *etc.*, and eventually human subjects.

Methods of introducing the vaccine may include oral, intracerebral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal or any other standard routes of immunization. The immune response of the test subjects can be analyzed by various approaches such as: the reactivity of the resultant immune serum to the

neurodegenerative disorder antigen, as assayed by known techniques, *e.g.*, immunosorbant assay (ELISA), immunoblots, radioimmunoprecipitations, *etc.*, or by protection of the immunized host against the neurodegenerative disorder.

As one example of suitable animal testing of a vaccine protective against neurodegenerative disorders and diseases, the vaccine of the invention may be tested in rabbits for the ability to induce an antibody response to the ND-associated antigen. Male specific-pathogen-free (SPF) young adult New Zealand White rabbits may be used. The test group each receives a fixed concentration of the vaccine. A control group receives an injection of 1 mM Tris-HCl pH 9.0 without the ND-associated antigen.

Blood samples may be drawn from the rabbits every one or two weeks, and serum analyzed for antibodies to the ND-associated protein. The presence of antibodies specific for the antigen may be assayed, *e.g.*, using an ELISA assay.

4.7.2 Monitoring of Effects During Immunotherapy

The effect of immunotherapy with ND-associated antigens on progression of neurodegerative diseases can be monitored by any methods known to one skilled in the art. In addition, cellular immunity may be monitored by methods including but not limited to measuring: a) delayed hypersensitivity as an assessment of cellular immunity; b) activity of cytolytic T-lymphocytes *in vitro*; c) levels of ND-associated antigen, *e.g.*, 42/43 β-amyloid.

Delayed hypersensitivity skin tests are also of great value in the overall immunocompetence and cellular immunity to an antigen. Inability to react to a battery of common skin antigens is termed anergy (Sato *et al.*, 1995, Clin. Immunol. Pathol. 74: 35-43).

Proper technique of skin testing requires that the antigens be stored sterile at 4°C, protected from light and reconstituted shorted before use. A 25- or 27-gauge needle ensures intradermal, rather than subcutaneous, administration of antigen. Twenty-four and 48 hours after intradermal administration of the antigen, the largest dimensions of both erythema and induration are measured with a ruler. Hypoactivity to any given antigen or group of antigens is confirmed by testing with higher concentrations of antigen or, in ambiguous circumstances, by a repeat test with an intermediate test.

In another optional method, the activity of cytolytic T-lymphocytes can be assessed *in vitro* using the following method. Eight x 10^6 peripheral blood-derived T lymphocytes isolated by the Ficoll-Hypaque centrifugation gradient technique, are restimulated with $4x10^4$ mitomycinC-treated cells in 3ml RPMI medium containing 10% fetal calf serum. In some experiments, 33% secondary mixed lymphocyte culture supernatant or IL-2, is included in the culture medium as a source of T cell growth factors.

In order to measure the primary response of cytolytic T-lymphocytes after immunization, T cells are cultured without the stimulator cells. In other experiments, T cells are restimulated with antigenically distinct cells. After six days, the cultures are tested for cytotoxicity in a 4 hour ^{51}Cr-release assay. The spontaneous ^{51}Cr-release of the targets should reach a level less than 20%. For the anti-MHC class I blocking activity, a tenfold concentrated supernatant of W6/32 hybridoma is added to the test at a final concentration of 12.5% (Heike M., *et al.*, J. Immunotherapy 15: 165-174).

In immunization procedures, the amount of immunogen to be used and the immunization schedule will be determined by a physician skilled in the art and will be administered by reference to the immune response and antibody titers of the subject.

The invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All references cited herein, including patent applications, patents, and other publications, are incorporated by reference herein in their entireties for all purposes.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an immunogenic amount of an antigenic molecule effective for treatment of or prevention of a neurodegenerative disorder, which antigenic molecule displays the antigenicity of an antigen associated with a neurodegenerative disorder, with the proviso that the antigenic molecule is not β amyloid.

2. The pharmaceutical composition of Claim 1, in which the antigenic molecule is purified.

3. The pharmaceutical composition of Claim 2, wherein the antigenic molecule is purified to apparent homogeneity, as viewed on an SDS-PAGE gel.

4. The pharmaceutical composition of Claim 1, in which the pharmaceutical carrier is an adjuvant.

5. The pharmaceutical composition of Claim 4, in which the antigenic molecule is purified.

6. The pharmaceutical composition of Claim 4, in which the adjuvant is a mineral salt, a mineral salt gel, an immunostimulatory molecule, a particulate or microparticulate adjuvant, or a mucosal adjuvant.

7. The pharmaceutical composition of Claim 6, in which the mineral salt is aluminum hydroxide, aluminum phosphate, or calcium phosphate.

8. The pharmaceutical composition of Claim 6, in which the immunostimulatory molecule is a cytokine, saponin, muramyl dipeptide or tripeptide derivative, CpG dinucleotide, CpG oligonucleotide, monophosphoryl Lipid A , or a polyphosphazene.

9. The pharmaceutical composition of Claim 6, in which the particulate adjuvant is an emulsion, liposome, virosome, cochleate, or an immune stimulating complex.

10. The pharmaceutical composition of Claim 1, which comprises a second pharmaceutical carrier, wherein at least one carrier is selected from the group consisting of a mineral salt, a mineral salt gel, an immunostimulatory molecule, a particulate adjuvant, a microparticulate adjuvant, and a mucosal adjuvant.

11. The pharmaceutical composition of Claim 1 in which the carrier is noncovalently associated with the antigenic molecule.

12. The pharmaceutical composition of Claim 1 in which the carrier is covalently associated with the antigenic molecule.

13. The pharmaceutical composition of Claim 1 in which the antigenic molecule is coupled to a universal helper.

14. The pharmaceutical composition of Claim 13 in which the universal helper is a tuberculin purified protein derivative, a tetanus toxin derivative, or a circumsporozoite protein derivative.

15. The pharmaceutical composition of Claim 1 in which the antigenic molecule is selected from the group consisting of an oligomeric Aβ complex, an ApoE4-Aβ complex, tau protein, a mutant amyloid precurser protein, a mutant of presenillin, α-synuclein, and a prion protein, or a fragment thereof.

16. The pharmaceutical composition of Claim 1 or 4 wherein the neurodegenerative disorder is Alzheimer's Disease, age-related loss of cognitive function, senile dementia, Parkinson's disease, amyotrophic lateral sclerosis, Wilson's Disease, cerebral palsy, progressive supranuclear palsy, Guam disease, Lewy body dementia, prion diseases, spongiform encephalopathies, Creutzfeldt-Jakob disease, polyglutamine diseases, Huntington's disease, myotonic dystrophy, Freidrich's ataxia, ataxia, Gilles de la Tourette's syndrome, seizure disorders, epilepsy, chronic seizure disorder, stroke, brain trauma, spinal cord trauma, AIDS dementia, alcoholism, autism, retinal ischemia, glaucoma, autonomic function disorder, hypertension, neuropsychiatric disorder, schizophrenia, or schizoaffective disorder.

17. The pharmaceutical composition of Claim 16 wherein the neurodegenerative disorder is Alzheimer's Disease.

18. A recombinant cell transformed with a nucleic acid comprising a nucleic acid sequence that is operably linked to a promoter, said nucleic acid sequence encoding a fusion protein that comprises an antigenic molecule operatively linked to a carrier protein, which antigenic molecule displaying the antigenicity of an antigen associated with a neurodegenerative disorder.

19. A recombinant cell transformed with (i) a first nucleic acid comprising a first nucleotide sequence that is operably linked to a first promoter and encodes an antigenic molecule that displays the antigenicity of an antigen associated with a neurodegenerative disorder, and (ii) a second nucleic acid comprising a second nucleic acid sequence that is operably linked to a second promoter and encodes a carrier protein, such that the antigenic molecule and the carrier protein are expressed within the cell and non-covalently associate with each other to form a complex that in sufficient amount is capable of eliciting an immune response to the antigenic molecule.

20. The recombinant cell of Claim 18 or 19 wherein the cell is a human cell.

21. The recombinant cell of Claim 18 or 19, wherein the antigenic molecule is an ApoE4-Aβ complex or a fragment thereof, tau protein or a fragment thereof, a mutant amyloid precurser protein or a fragment thereof, a mutant of presenillin or a fragment thereof, α-synuclein or a fragment thereof, a prion protein or a fragment thereof, or an oligomeric Aβ complex.

22. A pharmaceutical composition comprising the recombinant cell of Claim 18 or 19.

23. A method for preparing a fusion protein capable of eliciting an immune response against a neurodegenerative disorder, said method comprising:

a) culturing a recombinant cell transformed with a nucleic acid comprising a nucleotide sequence that is operably linked to a promoter and that encodes a fusion protein comprising an antigenic molecule operatively linked to a carrier, which antigenic molecule displays the antigenicity of an antigen associated with a neurodegenerative disorder with the proviso that the antigenic molecule is not β amyloid, under conditions such that the fusion protein is expressed by the cell; and

b) recovering the fusion protein from the cells.

24. A method for mixing the carrier with one or more antigenic molecules *in vitro*, which one or more antigenic molecules display the antigenicities of antigens associated with a neurodegenerative disorder, said method comprising:

a) incubating the antigenic molecule or molecules with a carrier protein under conditions and for a length of time sufficient for formation of the complex; and

b) isolating said complexes.

25. The method of Claim 23 or 24, wherein the one or more antigenic molecules is an ApoE4-Aβ complex or a fragment thereof, tau protein or a fragment thereof, a mutant amyloid precurser protein or a fragment thereof, a mutant of presenillin or a fragment thereof, α-synuclein or a fragment thereof, an oligomeric Aβ complex, or a prion protein or a fragment thereof.

26. A method for eliciting an immune response against an antigen associated with a neurodegenerative disorder in an individual comprising administering to the individual an antigenic molecule that displays the antigenicity of an antigen associated with a neurodegenerative disorder, with the proviso that the antigenic molecule is not β amyloid, and one or more pharmaceutically acceptable carriers in an amount effective to elicit an immune response.

27. The method of Claim 26, further comprising, before, concurrently, or after administration of the immunogenic complex, administering to the individual a composition comprising antigen presenting cells sensitized *in vitro* with a sensitizing amount of a second antigenic molecule, in which said second antigenic molecule shares at least one antigenic determinant with the first antigenic molecule.

28. A method of treating or protecting against a neurodegenerative disorder in an individual having a neurodegenerative disorder, or in whom prevention of a neurodegenerative disorder is desired, comprising administering to the individual a mixture of an antigenic molecule and a pharmaceutically acceptable carrier in an amount effective to treat or protect against said neurodegenerative disorder, wherein said antigenic molecule displays the antigenicity of an antigen associated with said neurodegenerative disorder, with the proviso that the antigenic molecule is not β amyloid.

29. The method of Claim 28, further comprising, before, concurrently, or after administration of the immunogenic complex, administering to the individual a composition comprising antigen presenting cells sensitized *in vitro* with a sensitizing amount of a second antigenic molecule and a pharmaceutically acceptable carrier, said second antigenic molecule sharing at least one antigenic determinant with the first antigenic molecule.

30. A method of treating or protecting against a neurodegenerative disorder in a subject having a neurodegenerative disorder or in whom prevention of a neurodegenerative disorder is desired comprising:

a) culturing a recombinant cell transformed with a nucleic acid comprising a nucleotide sequence that is operably linked to a promoter and that encodes a fusion protein comprising an antigenic molecule and a carrier, which antigenic molecule displays the antigenicity of an antigen associated with a neurodegenerative disorder, under conditions such that the fusion protein is expressed by the cell;

b) recovering the fusion protein from the cells; and

c) administering to the subject an amount of the fusion protein effective to treat or protect against the neurodegenerative disorder.

31. The method of Claim 28 or 30 wherein the neurodegenerative disorder is Alzheimer's Disease, age-related loss of cognitive function, senile dementia, Parkinson's disease, amyotrophic lateral sclerosis, Wilson's Disease, cerebral palsy, progressive supranuclear palsy, Guam disease, Lewy body dementia, prion diseases, spongiform encephalopathies, Creutzfeldt-Jakob disease, polyglutamine diseases, Huntington's disease, myotonic dystrophy, Freidrich's ataxia, ataxia, Gilles de la Tourette's syndrome, seizure disorders, epilepsy, chronic seizure disorder, stroke, brain trauma, spinal cord trauma, AIDS dementia, alcoholism, autism, retinal ischemia, glaucoma, autonomic function disorder, hypertension, neuropsychiatric disorder, schizophrenia, or schizoaffective disorder.

32. The method of Claim 31, wherein the neurodegenerative disorder is Alzheimer's Disease.

33. The method of Claim 31, wherein the antigenic molecule is an ApoE4-Aβ complex or a fragment thereof, tau protein or a fragment thereof, a mutant amyloid

precurser protein or a fragment thereof, a mutant of presenillin or a fragment thereof, α-synuclein or a fragment thereof, an oligomeric Aβ complex, or a prion protein or a fragment thereof.

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